Chromatin structure, DNA damage, DNA repair and cellular radiosensitivity

Woudstra, Elisabeth Cornelia

The radiosensitivity of tumours but also of normal, healthy tissue is known to vary from one patient to another even if they have the same kind of cancer. The reason(s) behind these differences in sensitivity to ionizing radiation are not known. In order to improve radiotherapy treatment it would be of great help to have a predictive assay that measures the sensitivity for ionizing radiation of a patients tumour and its surrounding normal tissue(s). So far, only the *in vitro* cellular survival of irradiated, isolated tumour cells was found to correlate well with the response of human tumours *in vivo*. However, this assay is not suitable as a predictive assay in the clinic, since not all (tumour) cells will be able to grow *in vitro* and also because the results obtained by this assay simply take too long.

To be able to find a predictive test for cellular ionizing radiation sensitivity, a lot of research is and has been done investigating the reasons behind the variations in cellular radiosensitivity. Ionizing radiation produces a variety of DNA damages of which the double strand break (DSB) is commonly accepted to be the major cause of cell death (described in §1.1). Cells respond to radiation by blocking their cell cycle (§1.2) in order to have time to repair the radiation induced DNA damages. The cell has various ways of repairing DNA damages by using different repair pathways for different kinds of damages (§1.3). Impairment of some of these pathways gives rise to enhanced sensitivity for ionizing radiation. Furthermore, it is thought that induction and repair of DNA damage is under influence of the structure of DNA attached to the protein nuclear matrix (chromatin structure) (§1.4).

In the current study (chapter 2 and 3), a human neuroblastoma cell line (HX142) and two human bladder carcinoma cell lines (RT112 and MGH-U1) were used that differ greatly in their intrinsic sensitivities to ionizing radiation. Importantly, the radiosensitive HX142 cells showed no evidence for a low DNA repair. With these cell lines, the hypothesis that the radiosensitivity of cells may be a result of a higher susceptibility to DNA damage induction was investigated. Firstly, the amount of DNA damage induced by ionizing radiation in these three cell lines was determined with the alkaline unwinding (AU) assay, the pulsed field gel electrophoresis (PFGE) assay, the alkaline comet assay, the halo assay, and a modified PFGE assay. Damage induction profiles were found to be the same for the three cell lines using the AU, comet and PFGE assays. However, consistent with literature data obtained using the non-denaturing elution (NDE) assay, the halo assay and the modified PFGE revealed steeper dose-response curves for initial damage for the

radiosensitive HX142 cells. This led to the conclusion that the amount of DNA damage per Gy per Da DNA is the same for the cell lines tested. It was further concluded that some aspect of chromatin structure affected the DNA damage detection in the assays that did reveal differences in the induction profiles between the cell lines. A model to describe these effects is presented in chapter 6.

The difference in chromatin structure could be the reason behind the variations in cellular radiosensitivity between the three cell lines. The results with the halo assay suggest that HX142 suffers a chromatin structure sensitivity at the level of DNA attached to the nuclear matrix (unstable Matrix Attachment Sites: MARs). In chapter 6 another model is presented illustrating how a difference in such a higher level of chromatin structure may affect cellular radiosensitivity. In short, a fragile chromatin structure (unstable MARs) may result in conversion of some otherwise repairable DSBs into irreparable DSBs. These irreparable DSBs then lead to radiosensitivity and can be visualized using only some assays.

The causes for the MAR instability of HX142 remain an enigma. Proteins responsible for DNA attachment to the nuclear matrix are not known. However, MAR instability in HX142 cells may be caused by differences in the protein composition of (sub) nuclear structures. We indeed found a lack of several major proteins in nuclear matrices isolated from HX142 cells. Attempts to identify these proteins, however, were yet unsuccessful (Chapter 3).

To see whether perhaps a fragile chromatin structure is a general feature for ionizing radiation sensitivity, we investigated 10 human tumour cell lines with a range in tumour cell radiosensitivity for DNA damage induction using the 'structure insensitive' PFGE and the 'structure sensitive' halo assay (chapter 4). No correlation could be found between DNA damage induction (PFGE or halo) and cellular survival, even when differences in DNA content and cell cycle distribution were taken into account. Thus, although other studies as well as studies reported in this thesis suggest that higher order chromatin structure fragility does enhance cellular radiosensitivity, it is not a dominant factor and therefore not of predictive value for (tumour) cell radiosensitivity on its own.

As discussed in chapter 1 and 6, the measurement of DNA repair as a single parameter to describe cellular radiosensitivity has also been of limited use to characterize cellular radiosensitivity. In fact, a picture is now emerging that neither one of the individual cellular characteristics after irradiation as mentioned in chapter 1 (signaling, chromatin structure sensitivity, DNA repair, cell cycle arrest, apoptotic response) is a dominant

factor useful for predictive purposes. This brings a period of optimism to a close but it also opens the mind for new directions of research regarding this subject. Rather than the individual measurements, we will require information on the combination of the various factors involved in the cellular response to radiation. This may seem unpractical using the current molecular and biochemical endpoints. However, with the increasing knowledge on the genes involved in these individual processes and rapidly developing techniques (such as gene arrays) a solution may become possible in the near future.

Support for the importance of chromatin structure at the level of MARs for cellular radiosensitivity has come from studies in which cells were treated with hyperthermia followed by X-rays. Hyperthermia is thought to inhibit the repair of radiation induced DNA damage due to protein denaturation and aggregation at the nuclear matrix, leading to higher rigidity of MARs. Yet, recently this hypothesis was challenged by the suggestion that heat-inactivation of proteins involved in the non-homologous end joining repair pathway was responsible for this effect. In chapter 5 it is shown, however, that heat enhances the radiosensitivity of cells that are either proficient or deficient in proteins that play major roles in the non-homologous recombination. Thus, inactivation of enzymes involved in non-homologous recombination pathway does not seem to play a crucial role in the enhancement of cellular radiosensitivity by hyperthermia. These results from chapter 5 imply, furthermore, that heat effects on MARs must have an impact on lesions other than those repaired by non-homologous recombination.

Finally, although a lot of attention is being paid to the genes controlling DNA repair, cell cycle arrest, and apoptosis following irradiation, little effort as yet is being put into research into genes controlling chromatin stability. Since the results described in this thesis reveal that chromatin structure can be an important factor for the radiosensitivity of cells, further research in this area, *e.g.* with mutant cell lines, is needed.